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PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant	:	Nabel et al.	Group Art Unit 1804
Serial No.	:	08/210,902	
Filed	:	March 21, 1994	
For	:	INHIBITION OF ARTERIAL SMOOTH MUSCLE CELL PROLIFERATION	
Examiner	:	J. Stone	

DECLARATION UNDER 37 C.F.R. § 1.132

Hon. Commissioner
of Patents and Trademarks
Washington, D.C. 20231

Dear Sir:

I, Elizabeth G. Nabel, declare and state:

1. I am an inventor in the above-identified application. I am familiar with the specification, claims and prosecution history thereof.

2. I received my Bachelor's degree in 1974 from St. Olaf College, Northfield, Minnesota. I received an M.D. degree in 1981 from Cornell University Medical College, New York, New York. From 1981-1982 I was an Intern in internal medicine; from 1982-1983 I was a Junior Resident; and from 1983-1984 I was a Senior Resident, all at Brigham and Women's Hospital (BWH), Boston, Massachusetts. From 1983-1984, I was a Research Fellow in Hypertension at BWH. From 1984-1987, I was a Clinical Fellow in Cardiology at BWH and from 1985-1987 I was a Research Fellow in Cardiology at BWH. From

1987-1991, I was an Assistant Professor of Internal Medicine, University of Michigan (UM), Ann Arbor, Michigan. From 1991-1994, I was an Associate Professor of Internal Medicine at UM. From 1992-1994, I was Interim Director of the Cardiovascular Research Center at UM. I am currently Professor of Internal Medicine and Director of the Cardiovascular Research Center at UM.

3. I am currently a member of the scientific advisory board of Vical, Inc., San Diego, California, and a consultant for RPI, Inc., Boulder, Colorado.

4. I have been engaged in cardiovascular research for the last 10 years.

5. I have authored approximately 60 publications in peer-reviewed journals including *Science*, *Proceedings of the National Academy of Sciences* and *Journal of Clinical Investigation*.

6. I belong to numerous professional societies including the American College of Physicians, the American College of Cardiology and the American Federation of Clinical Research.

7. I have read the Office Action dated August 15, 1994, in the above-identified application.

8. I understand that the PTO has stated that inhibition of restenosis in the pig model cannot be correlated to efficacy in humans. It is well accepted in the field that the pig model is the most correlatable predictor of human efficacy in treatments involving inhibition of restenosis. Within the last several years,

most investigators in this field have used the porcine model of vascular injury as an animal model.

9. The PTO also stated that it was unlikely that others would correlate the data presented in the specification with a therapeutic effect in view of the small sample size and individual species differences. It is common practice to investigate a smaller number of pigs compared to other smaller experimental animals. The studies described in the specification have now been performed in 30 pigs, 60 rabbits and 31 rats. The pig study is described by Ohno et al., *Science*, 265:781-784, 1994 (Exhibit D). The rabbit study is described in the enclosed unpublished data from my laboratory (Exhibit E). The rat study is described in the accompanying manuscript in press in *Mol. Med.* (Exhibit F). No significant species differences were observed in either intimal area or reduction in cellular proliferation. Similar numbers of animals have been used by other investigators.

10. The PTO stated, in reference to the Morishita et al. publication, that no effective pharmacological therapy for preventing restenosis in humans had been reported and that this failure may reflect previous difficulty in identifying appropriate drug targets. There are distinct differences between the adenoviral approach and the antisense approach of Morishita et al. In an antisense approach, the oligonucleotide must be delivered intracellularly to a sufficient number of cells to inhibit restenosis, since the oligonucleotide has its effects within a

single cell and not in adjacent cells. In contrast, our approach is effective in both transfected cells and in adjacent dividing cells by a bystander effect as defined below. The elimination of dividing cells can be achieved by expressing the tk gene in dividing vascular smooth muscle cells (SMCs). tk converts GCV into an active toxic form in SMCs. The subsequent incorporation of phosphorylated GCV into DNA results in cell death by inducing chain termination. In SMCs, a metabolite of phosphorylated GCV is diffusible to adjacent dividing SMCs, resulting in cell death in dividing SMCs not expressing tk. The end result is called a "bystander effect" which refers to the amplified response within a larger number of dividing SMCs. Thus, expression of tk in dividing SMCs following adenoviral infection and treatment with GCV kills adjacent dividing nontransduced SMCs through a bystander effect, thus amplifying the response and permitting the killing of a larger number of SMCs.

11. The PTO stated that the specification did not adequately disclose how to make and use adenoviral vectors in general, non-adenoviral vectors, vector-liposome or vector-ligand conjugates. The construction and use of these vectors is well known in the art. For example, the construction of other adenoviral vectors requires only a different cDNA insert. The adenovirus, restriction sites and ligation techniques are the same regardless of the cDNA to be expressed.

12. In regard to Lee et al., the Examiner stated that one could not extrapolate the results obtained by Applicants to other vectors since, according to Lee et al., "the level of gene transfer resulting from lipofection of the plasmid was far below that obtained with the Av1LacA4 vector." Lee et al. compared the efficiency of gene transfer of an adenoviral vector encoding a reporter gene and a plasmid encoding a reporter gene mixed with Lipofectin, a first generation cationic liposome manufactured by GIBCO BRL, Gaithersburg, Maryland. This preparation is not as efficient as current second or third generation cationic liposomes (Lipofectamine; Gibco BRL and DLRIE-DOPE, Vical, San Diego, CA, respectively.) Therefore, the statement of Lee et al. regarding efficiency of gene transfer does not reflect the use of current state of the art materials and, for that reason, carries little weight.

13. The PTO stated that the specification was not enabling for treatment of any and all "mechanical means" of injury, since different treatments may result in differences in arterial injury. Our claimed method applies to all mechanical means of injury, since all would result in subsequent vascular smooth muscle cell proliferation and intimal thickening. These are generic responses which occur after single or double injuries, whether by laser, balloon or stent.

14. In regards to Takeshita et al., where the effect of angioplasty on transfection efficiency of the marker gene

luciferase is measured, the treated lesions showed a reduction in stenosis, not "a reduction of restenosis," as contended by the PTO. The reduction is stenosis diameter following balloon injury is due to the injury itself, and is a common response to balloon angioplasty. Restenosis is defined as the recurrence of a stenosis, and was not evaluated by Takeshita et al.

15. I declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful, false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful, false statements may jeopardize the validity of the application or patent issuing therefrom.

Dated: 2/9/95 By: Elizabeth G. Nabel

Elizabeth G. Nabel, M.D.

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